



## **Methane production enhancement by an independent cathode in integrated anaerobic reactor with microbial electrolysis**

**Cai, Weiwei; Han, Tingting; Guo, Zechong; Varrone, Cristiano; Wang, Aijie; Liu, Wenzong**

*Published in:*  
Bioresource Technology

*Link to article, DOI:*  
[10.1016/j.biortech.2016.02.028](https://doi.org/10.1016/j.biortech.2016.02.028)

*Publication date:*  
2016

[Link back to DTU Orbit](#)

*Citation (APA):*  
Cai, W., Han, T., Guo, Z., Varrone, C., Wang, A., & Liu, W. (2016). Methane production enhancement by an independent cathode in integrated anaerobic reactor with microbial electrolysis. *Bioresource Technology*, 208, 13-18. <https://doi.org/10.1016/j.biortech.2016.02.028>

---

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

## Accepted Manuscript

Methane production enhancement by an independent cathode in integrated anaerobic reactor with microbial electrolysis

Weiwei Cai, Tingting Han, Zechong Guo, Cristiano Varrone, Aijie Wang, Wenzong Liu

PII: S0960-8524(16)30154-7

DOI: <http://dx.doi.org/10.1016/j.biortech.2016.02.028>

Reference: BITE 16078

To appear in: *Bioresource Technology*

Received Date: 26 November 2015

Revised Date: 3 February 2016

Accepted Date: 4 February 2016

Please cite this article as: Cai, W., Han, T., Guo, Z., Varrone, C., Wang, A., Liu, W., Methane production enhancement by an independent cathode in integrated anaerobic reactor with microbial electrolysis, *Bioresource Technology* (2016), doi: <http://dx.doi.org/10.1016/j.biortech.2016.02.028>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



# **Methane production enhancement by an independent cathode in integrated anaerobic reactor with microbial electrolysis**

Weiwei Cai<sup>1</sup>, Tingting Han<sup>1</sup>, Zechong Guo<sup>1</sup>, Cristiano Varrone<sup>3</sup>, Aijie Wang<sup>1,2\*</sup>

and Wenzong Liu<sup>2</sup>

1 State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (SKLUWRE, HIT), Harbin 150090, P.R.China

2 Key Laboratory of Environmental Biotechnology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China

3 Technical University of Denmark, Department of Chemical and Biochemical Engineering, Lyngby, Denmark

\*Corresponding author. E-mail address: waj0578@hit.edu.cn, wzliu@rcees.ac.cn

**\*Corresponding authors:**

**Dr. Aijie Wang**

State Key Laboratory of Urban Water Resource and Environment  
Harbin Institute of Technology  
Harbin, 150090, China

E-mail: [waj0578@hit.edu.cn](mailto:waj0578@hit.edu.cn); [wzliu@rcees.ac.cn](mailto:wzliu@rcees.ac.cn)

**Abstract**

Anaerobic digestion (AD) represents a potential way to achieve energy recovery from waste organics. In this study, a novel bioelectrochemically-assisted anaerobic reactor is assembled by two AD systems separated by anion exchange membrane, with the cathode placing in the inside cylinder (cathodic AD) and the anode on the outside cylinder (anodic AD). In cathodic AD, average methane production rate goes up to 0.070 mL CH<sub>4</sub>/mL reactor/day, which is 2.59 times higher than AD control reactor (0.027 m<sup>3</sup> CH<sub>4</sub>/m<sup>3</sup>/d). And COD removal is increased ~15% over AD control. When changing to sludge fermentation liquid, methane production rate has been further increased to 0.247 mL CH<sub>4</sub>/mL reactor/day (increased by 51.53% comparing with AD control). Energy recovery efficiency presents profitable gains, and economic revenue from increased methane totally self-cover the cost of input electricity. The study indicates that cathodic AD could cost-effectively enhance methane production rate and degradation of glucose and fermentative liquid.

**Keywords:** anaerobic digestion; methane; energy recovery; methanogenesis; cathode

## 1. Introduction

The current demand in renewable energy has boosted the applications of anaerobic wastewater treatment, which could provide biogas and at the same time produce lower sludge amount from wastewater. The conversion of fermentation products, such as amino acids (AA) and volatility fatty acids (VFA) to methane (by methanogens) is closely related to hydrolysis and fermentation of proteins and carbohydrates in AD (Chen et al., 2007; Eastman & Ferguson, 1981). Recently, a novel concept of microbial electrolysis cell (MEC) has been developed as a potential way to assist energy recycle from a wide range of organics, with a very high efficiency. Organic compounds are oxidized by anodic respiring bacteria and hydrogen is reduced on the Pt loading cathode with an small applied voltage ( $E_{app}=0.114V$  is theoretically needed, while  $E_{app}>0.130V$  is required due to overpotential(Call & Logan, 2008)). MEC have thus supplied a new pathway to effectively produce hydrogen from biomass, however cathode-induced methane production is still of major concern, due to the presence of methane-producing bacteria (Rozendal et al., 2006; Wagner et al., 2009).

Seemingly, microbial electrolysis cell has supplied a potential way to stimulate growth of hydrogenotrophs, which led to methane production by hydrogen consumption (or supplying electrons) at cathode (Villano et al., 2010). Recently, extra  $H_2$  or electrons from cathode have been proved to increase methane production and enhance stability in conventional AD reactor coupled MEC (which are frequently associated with hydrogenotrophic methanogenesis). Tao et al. (Bo et al., 2014), for instance, coupled anaerobic digestion in a single-chamber microbial electrolysis cell,

using a barrel-shape stainless steel as cathode, and reported a COD of acetate removal efficiency that increased from initial 56.5% (for the AD process) to 100% (for the coupled process) in 72 hours, while methane yield doubled. Zhang et al. (Zhang et al., 2015) operated a hybrid reactor with 0.3V applied voltage and obtained  $51 \pm 2.2\%$  COD removal, which was higher than AD reactor without voltage ( $44.3 \pm 1.2\%$ ), increasing at the same time hydrolysis of polysaccharides. Although hybrid reactors were tested for their ability to accelerate methane production and improve organics degradation (Zhang et al., 2015; Zhao et al., 2014), it is important to better understand the different roles played by anode and cathode in the AD system. Firstly, the most suitable substrates reported were different for anode respiring bacteria and cathode-involved bacteria (Liang et al., 2014). Secondly, reactions and/or products developed at the anode and cathode played different roles to methanogens, the presence of direct interspecies electron transfer between *Geobacter* and methanogens could primarily contribute to methane production (Zhao et al., 2015a), while cathode that was dominated by hydrogenotrophic methanogens take main responsible for the conversion from hydrogen/electrons to methane through biomass retention (De Vrieze et al., 2014; Siegert et al., 2015). However, single chamber integrated reactor consisted of anode and cathode was employed to enhance methane production in most studies, while the study concentrated on individual function of anode or cathode was still lack. Thus, a separated system between anode and cathode is expected to facilitate the evaluation of the relative contributions to AD improvement. The separated cathode chamber can avoid the effect of anode to methane production as the

presence of direct interspecies electron transfer (DIET) in the anode biofilm (Rotaru et al., 2014; Zhao et al., 2015a). Thus the contribution of cathode could be studied independently. Moreover, in previous studies, up-flow reactor could exhibit better performance either for anaerobic digestion in methane production (Zhao et al., 2015b) or bioelectrochemistry system in current generation (Wang et al., 2012). Therefore, the integrated reactor was designed to couple up-flow construction and separated anode/cathode in order to achieve better performance and eliminate irrelevant effects.

The objective of this study was to investigate the contribution of cathode in methane production and organic removal efficiency. A novel reactor, two integrated ADs was constructed and connected by bioanode and biocathode independently to evaluate the effect on methane acceleration and COD removal. Glucose and synthetic fermentation liquid were used to evaluate the performance in new reactor, including enhancement and stability of methane production.

## 2. Material and methods

### 2.1 Bioreactor setup

A new integrated anaerobic reactor made of polymethylmethacrylate was tested, by integrated a bioelectrochemical system between two AD systems, which were separated by anion exchange membrane (AEM, Ultrex CMI-7000, Membranes International, U.S.) into anodic AD and cathodic AD. The cathode was placed in the inner cylinder and the anode was in the outer cylinder. The total volume was 1.2 L, including 700 mL of inner and 500 mL of external. The cathode was made of stainless steel mesh, which was placed close to the AEM, in the inside cylinder. The anode was made of carbon brush, placed in the external tube. A 0.8 V applied voltage was added between anode and cathode, based on our previous study (Linji et al., 2013). A control reactor was operated synchronously but without external voltage.

Acetate (1.5 g/L) was used as the sole carbon source in the anode chamber. Glucose (2.5 g/L in 50 mM PBS) was used as carbon source in the cathodic AD for methane production and for reactor performance evaluation. Subsequently, sludge fermentation liquid (SFL) was used as complex carbons for application test. The main characteristics of sludge fermentation liquid was 750 mg/L acetate, 150 mg/L propionate, 200 mg/L n-butyrate, 300 mg/L polysaccharide and 650 mg/L protein. The mixture of SFL and 50 mM PBS (50:50, V:V) was used as influent to cathodic AD.

### 2.2 Bioreactor operation

Acetate was used as carbon source for anodic AD throughout the whole experiment.

During startup, all reactors were inoculated by waste activity sludge taken from



aeration tank of the Taiping Municipal Wastewater Treatment Plant (Harbin, Heilongjiang Province, China). Glucose was firstly used as simple carbon source for cathode AD. Two integrated reactors (0.8 V applied voltage) and one control reactor (without applied voltage) were operated in continuous mode at a hydraulic retention time of 24h. All reactors were operated at room temperature of 25 °C for 30 days. To evaluate bioelectrochemical contribution to methane production in integrated reactors, the applied voltage of one reactor was cut for 7 days as operational control and recovered applied voltage back. Finally, sludge fermentation liquid was used as complex carbons for cathodic AD for organic removal and methane production for another 26 days.

### 2.3 Measurement and calculation

Agilent gas chromatography (GC) was utilized to analyze the composition of VFAs (Zhou et al., 2013b). The determination of SCOD, TCOD, carbohydrate and protein was performed as described previously (Zhou et al., 2013a). Voltage and current were recorded every 10 min, using a multimeter (model 2700; Keithley Instruments, the USA). Gases (hydrogen, carbon dioxide, methane) were analyzed by TCD GC (Fuli GC9790II, Zhejiang analytical instrument Inc., China).

The contribution of current was calculated as theoretical methane production.

$$\frac{It}{nF} \cdot V_m = V_{CH_4}$$

where  $I$  is the current value,  $t$  the time,  $n=8$  represents 8 mol electrons that can be transfer into 1mol of methane,  $F=96485$  C/mol is Faraday's constant,  $V_m$  (22.4 L/mol) is the gas constant, whereas  $V_{CH_4}$  represents the volume of methane.

The revenue from methane was calculated as follows:

$$R_{\text{methane}} = P_e \frac{\frac{Y_{\text{methane}}}{V_m} \Delta H}{3600} \eta$$

with  $P_e = 0.06892$  €/kW·h, representing the standard price of electricity (referenced from average standards in 100 cities of China),  $R_{\text{methane}}$  is the revenue from methane expressed as €/m<sup>3</sup> reactor/d,  $Y_{\text{methane}}$  (mL CH<sub>4</sub>/mL reactor/day) represents the methane production rate of the reactor,  $\Delta H = 890$  kJ/mol is the calorific value of methane.

$V_m = 22.4$  L/mol.  $\eta = 35\%$  (Rabaey & Verstraete, 2005), is the electrical efficiency with a combustion engine as converter.

### 3. Results and Discussion

#### 3.1 Enhancement of methane production in integrated reactors with glucose as substrate

In the first stage (0-7 d), methane was produced quickly during the initial days in integrated reactors, while there was no methane detected in the AD control reactor without applied voltage till the 7<sup>th</sup> day (Fig. 1A). Methane production rate in the hybrid reactor increased substantially from 0.01 m<sup>3</sup>/m<sup>3</sup> reactor/d (first day) to 0.06 m<sup>3</sup>/m<sup>3</sup> reactor/d (14<sup>th</sup> day), and stayed at 0.07 m<sup>3</sup>/m<sup>3</sup> reactor/d until the end of this stage (15-37 d). AD control exhibited a lower methane production rate (on average ~0.027 m<sup>3</sup>/m<sup>3</sup> reactor/d), with no increase throughout the operation time (15-37 d). Average methane production rate of the hybrid reactor was thus improved about 2.59 times (increased by 159.26%) compared to the AD reactor. Methane production rate was enhanced thanks to the independent cathode.

At the end of the experiment (on 30<sup>th</sup> day), reactor 1<sup>#</sup> was switched from closed circuit to open. Methane production rate decreased sharply during the following days, reaching on average 0.031 m<sup>3</sup>/m<sup>3</sup> reactor/d, which was close to the performance of the AD control reactor. However, methane production rate in reactor 2<sup>#</sup> (with applied voltage) was still higher than AD control, seemingly due to the contribution of the cathode.

The fluctuation of current was consistent with methane production, showing a similar trend (Fig. 1B). The hybrid reactors started successfully in two weeks. A clear increase in current occurred at the 10<sup>th</sup> day, with a concomitant increase of methane production rate, thus suggesting a potential relation between current and methane

production rate. At the stage of stable methane generation, the peak current kept steady around 10.53 mA (1<sup>#</sup>) and 8.67 mA (2<sup>#</sup>). When the external voltage was cut, a drop of methane production rate was detected from 0.070 m<sup>3</sup>/m<sup>3</sup> reactor/d to 0.031 m<sup>3</sup>/m<sup>3</sup> reactor/d.

Wang and colleagues proved that external voltage significantly regulated hydrogen production on cathode in single chamber MEC (Wang et al., 2009), however, hydrogen was quickly consumed for methane production in an AD system. Lower cathode potential ( $\leq -1000$  mV with calomel electrode in our study) brought from external voltage could probably stimulate the growth of hydrogenotrophic methanogens (Hirano et al., 2013). Recent researches also showed that hydrogenotrophic methanogens could accept electrons from both, electrode (Lohner et al., 2014) or hydrogen (Stams & Plugge, 2009). Hydrogenotrophic metabolism was considered as a key process to capture hydrogen (or electrons) to enhance methane production (Villano et al., 2011; Xu et al., 2014). In our study, the increase in methane production could be ascribed to the positive effect of the cathode.

### **3.2 Methane production with sludge fermentation liquid as substrate**

After operating the reactor with glucose for one month, sludge fermentation liquid (containing polysaccharide, protein and VFAs) was used as carbon source for the cathode AD, both in 2<sup>#</sup> integrated reactor and the control reactor. The complex substrate primarily caused a shock to both reactors. It took about 10 days for the systems to get high methane production. However, a substantial increase of gas production could be achieved. Following the increase of current, going from 8.67 mA

(glucose, fig. 1B) to 9.65 mA (Fig. 2), methane in integrated reactors markedly rose from day 10 and reached 0.247 mL CH<sub>4</sub>/mL reactor/day. Methane production rate of control reactor rose from day 11 to day 13 and finally stabilized at an average of 0.163 mL CH<sub>4</sub>/mL reactor/day. Obviously, higher methane production was obtained when using sludge fermentation liquid compared to glucose. In integrated reactor, the methane production rate of complex substrate gave methane production rate 3.53 fold higher than that of glucose. Meanwhile, a 6.04-fold increase in methane production rate was also achieved with sludge fermentation liquid in control reactor. As the acetate was main substrate for methanogens to produce methane, the sludge fermentation liquid contained acetate, propionate, butyrate was favourable for the growth of methanogens and methane generation compared with glucose (Costa & Leigh, 2014). Besides, this increase in methane production suggested the potential for methane production from waste activity sludge after shorten fermentation, which was accordant with our previous study (Zhou et al., 2013b). Anyway, it was meaningful to find that the cathode was also able to boost methane generation in this integrated reactor, indicating a good ability to degrade complex organic compounds in cathodic AD. Importantly, a relatively stable current rather than methane production after changing to complex substrate, indicated that electron transfer was mainly determined by availability of anodic substrate for anode bacteria (Cheng & Logan, 2007; Kiely et al., 2011; Wang et al., 2010). In theory, if all current contributed to produce methane in cathodic AD, average methane production rate would reach 0.028 mL CH<sub>4</sub>/mL reactor/day. Even considering methane (0.028 m<sup>3</sup> CH<sub>4</sub>/m<sup>3</sup> reactor/d) from AD control

reactor, the sum would have been only  $0.191 \text{ m}^3 \text{ CH}_4/\text{m}^3 \text{ reactor/d}$ , which was lower than  $0.247 \text{ m}^3/\text{m}^3 \text{ reactor/d}$  obtained in the integrated reactor. Therefore, the increased methane production might be ascribed to the complex microbial networks constructed by different microorganisms, especially hydrogenotrophic methanogens, interacting with other bacteria (Fernandez et al., 2000; Sasaki et al., 2011). In recent study, lower redox potential (lower than  $-800\text{mV}$  in our experiment) could enhance methane-producing activity with hydrogenotrophic methanogens, indicating cathodic methanogens community owned high cell density (Hirano et al., 2013). Hence, it seems to be reasonable that biomass on the electrode might have contributed to enhance methane production rate in the integrated reactor. Moreover, almost all of the methane originated from substrate, electric current could stimulate the microbial metabolism to enhance the degradation of substrate that was accordingly proved by the higher COD removal efficiency in integrated reactor. Therefore, input of a small amount of electric energy as reducing power in microorganisms would effectively increase total microbial growth and methane production (Sasaki et al., 2011).

### 3.3 Substrate degradation in integrated reactor

In anaerobic digestion processes, VFAs accumulate as end products in reactors and finally are degraded to produce methane. In order to evaluate AD performance, concentration of VFAs in terms of acetate, propionate, n-butyrate, iso-butyrate, n-valerate and iso-valerate, were determined in all reactors. As shown in figure 3, when glucose was used as substrate, the COD removal efficiency went up to 28.7% in the integrated reactor, which was close to the AD control reactor. At the beginning

(before methane was produced), a slight accumulation of VFAs was detected. From the 10<sup>th</sup> day to the 20<sup>th</sup>, propionate and butyrate decreased in both reactors, while acetate declined noticeably in hybrid reactor only. The change of VFAs in the integrated reactor occurred with a simultaneous methane production rate increase, suggesting that the two phenomena could be related closely.

From day 10 to 31, COD removal efficiency in hybrid reactor ranged between 40%~60%, which was clear higher than that of AD (which was below 40%). After substrate was changed to sludge fermentation liquid, VFAs were rapidly degraded. The n-butyrate, iso-butyrate, n-valerate and iso-valerate exhibited similar degradation trends in both reactor, while a clear decrease of acetate and propionate concentration was visible in hybrid reactor only. The final concentration of acetate and propionate in the integrated reactor kept stable around 1000 mg/L and 400 mg/L, respectively. The removal efficiency of COD was 49.62%, which was close to the result using glucose. However, the organic loading rate was increased to 3.8 kg/m<sup>3</sup>/d using sludge fermentation liquid, which was higher than the 2.5 kg/m<sup>3</sup>/d of glucose.

Methane production rate was significantly enhanced when substrate changed from glucose to sludge fermentation liquid (for the cathode). A similar COD removal efficiency was obtained in the cathodic AD system, though organic loading was increased by 52% in sludge fermentation liquid. Methane production yield of the integrated reactor was increased by  $0.031 \pm 0.007$  m<sup>3</sup>/kgCOD compared to control AD using glucose, and increased by  $0.025 \pm 0.010$  m<sup>3</sup>/kgCOD when using sludge fermentation liquid. This suggested that contribution of the bioelectrochemical system

to the total electron recovery worked stably on various cathodic substrates, but it was mainly determined by anodic AD, most probably including anodic substrate and biofilm formation (Liu et al., 2010). Noticeably, methane yield was increased from 0.054 m<sup>3</sup>/kgCOD of glucose to 0.122 m<sup>3</sup>/kgCOD of sludge fermentation liquid. Previous observations also indicated that the presence of fermentable byproducts such as acetate, propionate, butyrate could be rapidly consumed to improve the conversion from organic matters to methane (Rozendal et al., 2008).

It is presumable that the introduction of electrolysis cathode could contribute to stimulate microbial metabolism and organic degradation, which resulted in methane production enhancement in hybrid reactor (Thrash & Coates, 2008).

Bioelectrochemical system integrated with anaerobic digestion exhibited stable performance with both, glucose as well as sludge fermentation liquid as substrate, which implies that BES could be applied to assist methane production from AD in future application. It was prospective to use fermentation wastewater as alternative carbon source to produce methane in anaerobic reactor.

### **3.4 Input energy recovery efficiency and economic analysis**

Promotion of methane production rate was induced by the introduction of BES that drive hydrogen evolution at cathode, however, it is essential to evaluate the input energy recovery efficiency as additional cost. Input energy recovery efficiency was calculated when stable performance was obtained in hybrid reactor. The results showed a clear distinction, depending on the different substrate. The overall energy exceed 100% and reached up to 200% when glucose was used as substrate, whereas



more than 400% input energy recovery efficiency was achieved with sludge fermentation liquid. As shown in Figure 4, one hundred percentage energy covered electric input totally, which indicated that the segment exceeding 100% could be considered as revenue, resulted from enhancement of methane production.

Considering an electrical consumption of  $0.19 \times 10^{-3}$  kW·h (with an average cost of 0.019 €/m<sup>3</sup>/day), overall electricity cost was 0.06982 €/ kW·h. In hybrid reactor, methane production rate (0.247 mL CH<sub>4</sub>/mL reactor/day) corresponded to 0.067 €/m<sup>3</sup>reactor/d, when using sludge fermentation liquid. The additional revenue in the hybrid reactor was 0.023 €/m<sup>3</sup>reactor/d, considering 0.044 €/m<sup>3</sup>reactor/d for the AD reactor (and based on 0.163 mL CH<sub>4</sub>/mL reactor/day).

When glucose was used as substrate, the average methane production rate was 0.07 mL CH<sub>4</sub>/mL reactor/day (integrated reactor) and 0.027 mL CH<sub>4</sub>/mL reactor/day (AD), respectively, corresponding to 0.019 €/ m<sup>3</sup>reactor/d and 0.07 €/ m<sup>3</sup>reactor/d revenue returned during wastewater treatment. It is interesting that the lower additional revenue ( $0.012 \text{ €} = 0.019 - 0.07 \text{ mL CH}_4/\text{mL reactor/day}$ ) was obtained with glucose. According to the economic analysis, sludge fermentation liquid resulted to be more viable for integrated reactor. Furthermore, economic analysis revealed that introduction of BES totally self-covered the cost caused by electric input, using complex substrate.

#### 4. Conclusion

The novel anaerobic digestion reactor enhanced methane production regardless of the substrate (glucose or synthesis fermentation liquid). The maximum methane production in integrated reactor was 0.247 mL CH<sub>4</sub>/mL reactor/day, which was significant higher than that of control AD reactor (0.163 mL CH<sub>4</sub>/mL reactor/day). Independent cathode played an important role in enhancing methane production, by stimulating microbial metabolism and organic degradation especially fermentation liquid. Importantly, overall input energy recovery efficiency exceed 100% and the additional revenue from increased methane could cover electric input cost, indicating the feasibility of using independent cathode to enhance methane production from wastewater in future application.

#### Acknowledgements

This research was supported by National Science Foundation for Distinguished Young Scholars (Grant No. 51225802), National Natural Science Foundation of China (no. 51208496), Project 135 of Chinese Academy of Sciences (No. YSW2013B06), and ‘Hundred Talents Program’ of the Chinese Academy of Sciences, and International S&T cooperation program (S2015GR1012).

## Reference

- 1 Bo, T., Zhu, X., Zhang, L., Tao, Y., He, X., Li, D., Yan, Z. 2014. A new upgraded biogas production process: Coupling microbial electrolysis cell and anaerobic digestion in single-chamber, barrel-shape stainless steel reactor. *Electrochem Commun*, 45, 67-70.
- 2 Call, D., Logan, B.E. 2008. Hydrogen production in a single chamber microbial electrolysis cell lacking a membrane. *Environ Sci Technol*, 42, 3401-3406.
- 3 Chen, Y., Jiang, S., Yuan, H., Zhou, Q., Gu, G. 2007. Hydrolysis and acidification of waste activated sludge at different pHs. *Water Res*, 41, 683-9.
- 4 Cheng, S., Logan, B.E. 2007. Sustainable and efficient biohydrogen production via electrohydrogenesis. *Proc Natl Acad Sci U S A*, 104, 18871-3.
- 5 Costa, K.C., Leigh, J.A. 2014. Metabolic versatility in methanogens. *Curr Opin Biotechnol*, 29, 70-5.
- 6 De Vrieze, J., Gildemyn, S., Arends, J.B., Vanwonterghem, I., Verbeken, K., Boon, N., Verstraete, W., Tyson, G.W., Hennebel, T., Rabaey, K. 2014. Biomass retention on electrodes rather than electrical current enhances stability in anaerobic digestion. *Water Res*, 54, 211-21.
- 7 Eastman, J.A., Ferguson, J.F. 1981. Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *Journal (Water Pollution Control Federation)*, 352-366.
- 8 Fernandez, A.S., Hashsham, S.A., Dollhopf, S.L., Raskin, L., Glagoleva, O., Dazzo, F.B., Hickey, R.F., Criddle, C.S., Tiedje, J.M. 2000. Flexible community structure correlates with stable community function in methanogenic bioreactor communities perturbed by glucose. *Appl Environ Microb*, 66, 4058-4067.
- 9 Hirano, S., Matsumoto, N., Morita, M., Sasaki, K., Ohmura, N. 2013. Electrochemical control of redox potential affects methanogenesis of the hydrogenotrophic methanogen *Methanothermobacter thermautotrophicus*. *Lett Appl Microbiol*, 56, 315-21.
- 10 Kiely, P.D., Rader, G., Regan, J.M., Logan, B.E. 2011. Long-term cathode performance and the microbial communities that develop in microbial fuel cells fed different fermentation endproducts. *Bioresource Technol*, 102, 361-366.
- 11 Liang, B., Cheng, H., Van Nostrand, J.D., Ma, J., Yu, H., Kong, D., Liu, W., Ren, N., Wu, L., Wang, A., Lee, D.J., Zhou, J. 2014. Microbial community structure and function of nitrobenzene reduction biocathode in response to carbon source switchover. *Water Res*, 54, 137-48.
- 12 Linji, X., Wenzong, L., Yining, W., Aijie, W., Shuai, L., Wei, J. 2013. Optimizing external voltage for enhanced energy recovery from sludge fermentation liquid in microbial electrolysis cell. *INT J HYDROGEN ENERG*, 38, 15801-15806.
- 13 Liu, W., Wang, A., Cheng, S., Logan, B.E., Yu, H., Deng, Y., Nostrand, J.D.V., Wu, L., He, Z., Zhou, J. 2010. Geochip-based functional gene analysis of anodophilic communities in microbial electrolysis cells under different operational modes. *Environ Sci Technol*, 44, 7729-7735.
- 14 Lohner, S.T., Deutzmann, J.S., Logan, B.E., Leigh, J., Spormann, A.M. 2014. Hydrogenase-independent uptake and metabolism of electrons by the archaeon *Methanococcus maripaludis*. *ISME J*, 8, 1673-81.
- 15 Rabaey, K., Verstraete, W. 2005. Microbial fuel cells: novel biotechnology for energy generation. *Trends Biotechnol*, 23, 291-8.
- 16 Rotaru, A.-E., Shrestha, P.M., Liu, F., Shrestha, M., Shrestha, D., Embree, M., Zengler, K., Wardman, C., Nevin, K.P., Lovley, D.R. 2014. A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to *Methanosaeta* for the reduction of

- carbon dioxide to methane. *Energy Environ. Sci.*, 7, 408-415.
- 17 Rozendal, R., Hamelers, H., Euverink, G., Metz, S., Buisman, C. 2006. Principle and perspectives of hydrogen production through biocatalyzed electrolysis. *INT J HYDROGEN ENERG*, 31, 1632-1640.
  - 18 Rozendal, R.A., Hamelers, H.V., Rabaey, K., Keller, J., Buisman, C.J. 2008. Towards practical implementation of bioelectrochemical wastewater treatment. *Trends Biotechnol*, 26, 450-459.
  - 19 Sasaki, K., Hirano, S., Morita, M., Sasaki, D., Matsumoto, N., Ohmura, N., Igarashi, Y. 2011. Bioelectrochemical system accelerates microbial growth and degradation of filter paper. *Appl Microbiol Biotechnol*, 89, 449-55.
  - 20 Siegert, M., Yates, M.D., Spormann, A.M., Logan, B.E. 2015. Methanobacterium Dominates Biocathodic Archaeal Communities in Methanogenic Microbial Electrolysis Cells. *ACS SUSTAIN CHEM ENG*, 3, 1668-1676.
  - 21 Stams, A.J., Plugge, C.M. 2009. Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *NAT REV MICROBIOL*, 7, 568-577.
  - 22 Thrash, J.C., Coates, J.D. 2008. Review: direct and indirect electrical stimulation of microbial metabolism. *Environ Sci Technol*, 42, 3921-3931.
  - 23 Villano, M., Aulenta, F., Ciucci, C., Ferri, T., Giuliano, A., Majone, M. 2010. Bioelectrochemical reduction of CO(2) to CH(4) via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture. *Bioresource Technol*, 101, 3085-90.
  - 24 Villano, M., Monaco, G., Aulenta, F., Majone, M. 2011. Electrochemically assisted methane production in a biofilm reactor. *J POWER SOURCES*, 196, 9467-9472.
  - 25 Wagner, R.C., Regan, J.M., Oh, S.E., Zuo, Y., Logan, B.E. 2009. Hydrogen and methane production from swine wastewater using microbial electrolysis cells. *Water Res*, 43, 1480-8.
  - 26 Wang, A., Liu, W., Cheng, S., Xing, D., Zhou, J., Logan, B.E. 2009. Source of methane and methods to control its formation in single chamber microbial electrolysis cells. *INT J HYDROGEN ENERG*, 34, 3653-3658.
  - 27 Wang, A., Liu, W., Ren, N., Zhou, J., Cheng, S. 2010. Key factors affecting microbial anode potential in a microbial electrolysis cell for H<sub>2</sub> production. *INT J HYDROGEN ENERG*, 35, 13481-13487.
  - 28 Wang, A.J., Cui, D., Cheng, H.Y., Guo, Y.Q., Kong, F.Y., Ren, N.Q., Wu, W.M. 2012. A membrane-free, continuously feeding, single chamber up-flow biocatalyzed electrolysis reactor for nitrobenzene reduction. *J Hazard Mater*, 199-200, 401-9.
  - 29 Xu, H., Wang, K., Holmes, D.E. 2014. Bioelectrochemical removal of carbon dioxide (CO<sub>2</sub>): An innovative method for biogas upgrading. *Bioresource Technol*, 173, 392-398.
  - 30 Zhang, J., Zhang, Y., Quan, X., Chen, S. 2015. Enhancement of anaerobic acidogenesis by integrating an electrochemical system into an acidogenic reactor: effect of hydraulic retention times (HRT) and role of bacteria and acidophilic methanogenic Archaea. *Bioresource Technol*, 179, 43-9.
  - 31 Zhao, Z., Zhang, Y., Chen, S., Quan, X., Yu, Q. 2014. Bioelectrochemical enhancement of anaerobic methanogenesis for high organic load rate wastewater treatment in a up-flow anaerobic sludge blanket (UASB) reactor. *Sci Rep*, 4, 6658.
  - 32 Zhao, Z., Zhang, Y., Wang, L., Quan, X. 2015a. Potential for direct interspecies electron transfer in an electric-anaerobic system to increase methane production from sludge digestion. *Sci Rep*, 5, 11094.

- 33 Zhao, Z., Zhang, Y., Woodard, T.L., Nevin, K.P., Lovley, D.R. 2015b. Enhancing syntrophic metabolism in up-flow anaerobic sludge blanket reactors with conductive carbon materials. *Bioresource Technol*, 191, 140-5.
- 34 Zhou, A., Guo, Z., Yang, C., Kong, F., Liu, W., Wang, A. 2013a. Volatile fatty acids productivity by anaerobic co-digesting waste activated sludge and corn straw: Effect of feedstock proportion. *J. Biotechnol*.
- 35 Zhou, A., Yang, C., Guo, Z., Hou, Y., Liu, W., Wang, A. 2013b. Volatile fatty acids accumulation and rhamnolipid generation in situ from waste activated sludge fermentation stimulated by external rhamnolipid addition. *BIOCHEM ENG J*, 77, 240-245.

Figure legends:

Figure 1 A: Methane production rate in all reactors with glucose as substrate, —○— 1# Integrated reactor, —△— 2# Integrated reactor, —□— AD.

B: Current value of integrated reactor . ● 1# Integrated reactor current, ■ 2# Integrated reactor current.

Figure 2 Methane production rate and current value in integrated reactor and AD reactor with sludge fermentation liquid as substrate, —△— 2# Integrated reactor, —□— AD, —●— 2# Integrated reactor Current.

Figure 3 VFAs concentration analysis and COD removal efficiency in cathode chamber.

A: AD control reactor; B: 2# integrated reactor.

- acetate
- ◆— propionate
- n-butyrate
- iso-butyrate
- ▲— n-valerate
- ▼— iso-valerate
- COD removal efficiency

Figure 4 Input energy recovery efficiency and economic analysis of integrated reactor.

- Energy efficiency: Glucose
- Energy efficiency: Sludge fermentation liquid
- Revenue: Glucose
- Revenue: Sludge fermentation liquid

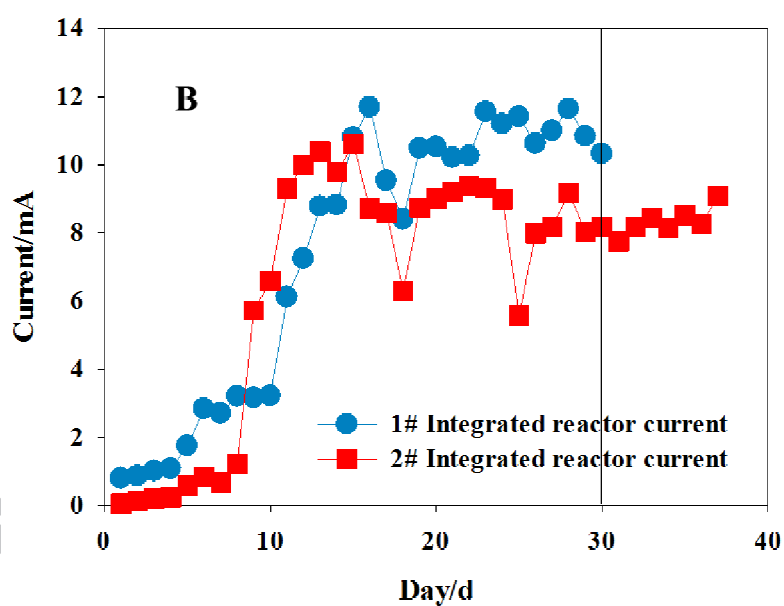
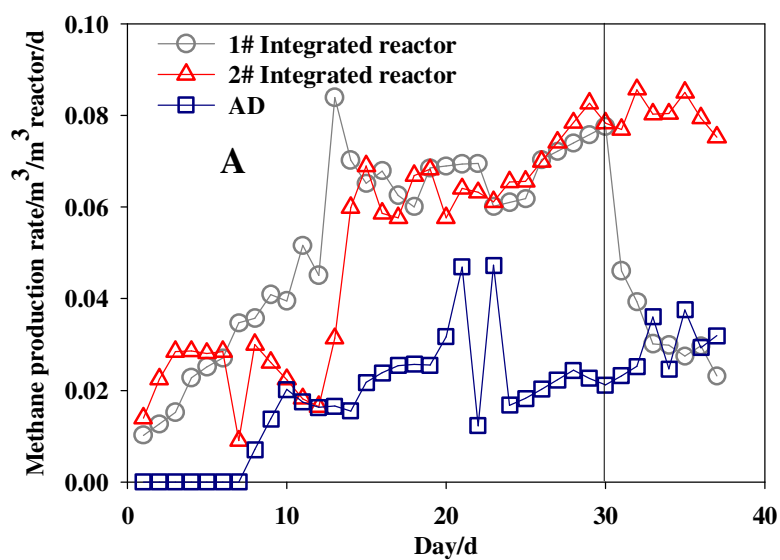


Figure 1 A: Methane production rate in all reactors with glucose as substrate;  
 B: Current value of integrated reactor.

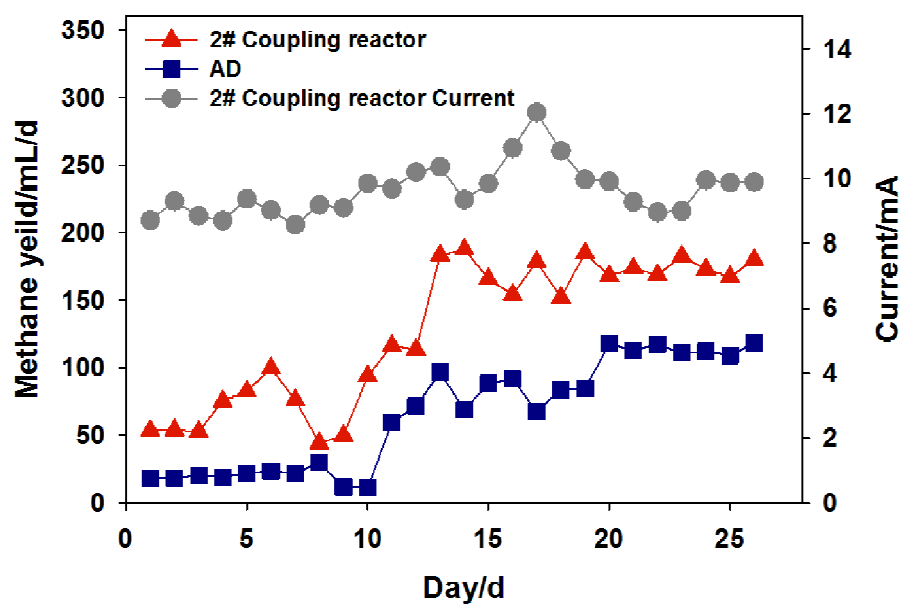


Figure 2 Methane production rate and current value in integrated reactor and AD reactor with sludge fermentation liquid as substrate.



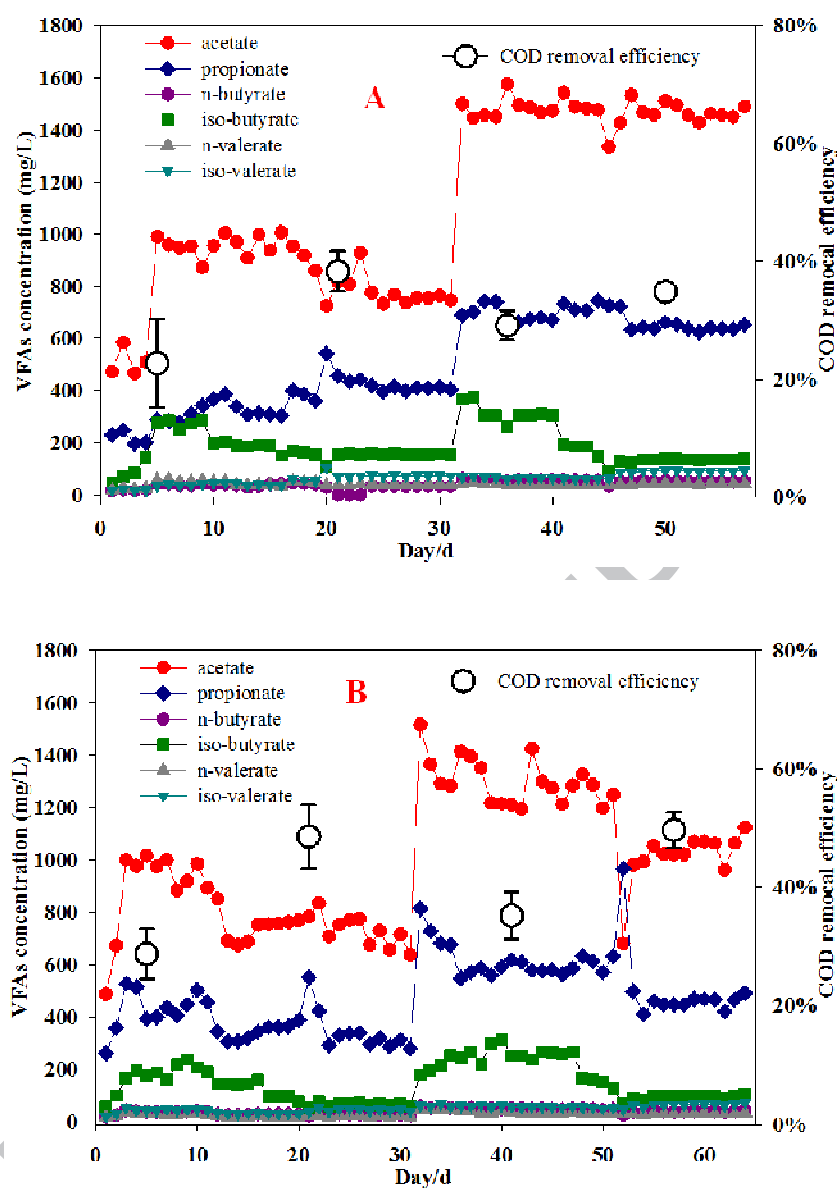


Figure 3 A: VFAs concentration analysis and COD removal efficiency in cathode chamber.

A: AD control reactor; B: 2# integrated reactor.

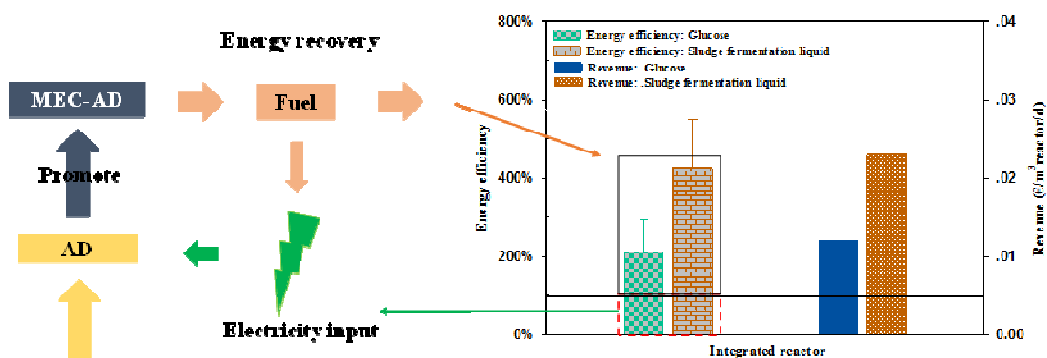
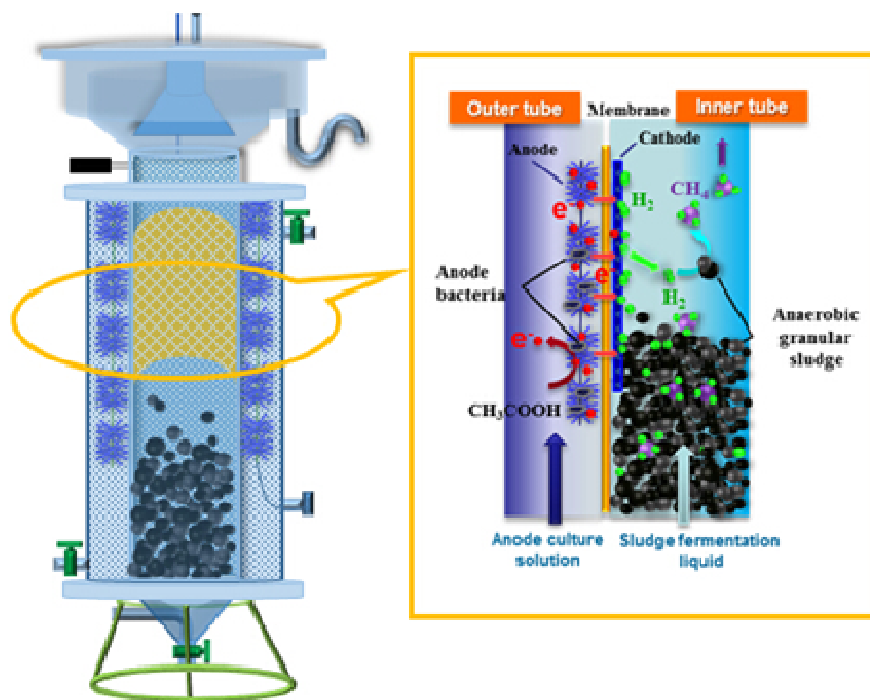


Figure 4 Input energy recovery efficiency and economic analysis of integrated reactor.

Graphical abstract



Integrating microbial electrolysis cell and anaerobic bioreactor to enhance methane production from organics

#### Highlights

1. Methane production rate was increased in cathodic anaerobic digestion process.
2. Independent cathode mainly contributed to the increase of methane production.
3. Fermentative liquid was efficiently utilized in integrated reactor.
4. Economic revenue can self-cover the cost of input electricity.